Comparative morphology and mechanical properties of the lepidotrichia of climbing and non-climbing Hawaiian gobioid fishes

by

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Abstract. – Many gobioid fishes from tropical islands exhibit amphidromous life histories, in which juveniles migrate from the ocean to upstream freshwater habitats. However, upstream reaches are blocked by waterfalls on many islands. In some species, juveniles climb these waterfalls with the aid of an adhesive sucker formed from fusion of the pelvic fins. Adults of some species may also climb waterfalls to return upstream if they are dislodged by pulses of high flow. Several structural features of gobies have been examined to assess how they contribute to successful climbing. In this study, we compared the morphology and flexural stiffness of lepidotrichia from the pelvic and caudal fins for four of the five native species of Hawaiian stream fishes, including both climbing and non-climbing taxa, to evaluate how the structure and mechanical properties of the fin skeleton might facilitate climbing success. We found that pelvic lepidotrichia were uniformly more flexible than caudal lepidotrichia, and that the lepidotrichia of climbing taxa were shorter and more flexible than those of non-climbing taxa. These lepidotrichial properties may allow climbing taxa to maximize the pressure differentials and adhesive strength that can be achieved with gobioid suckers, without requiring excessively large suckers that could incur increased hydrodynamic drag. These results highlight how variation in skeletal mechanical properties can contribute to the remarkable functional capacities and ecological distribution of tropical island stream fishes.

Key words
Gobioidei
Fin ray
Lepidotrichia
Adhesive sucker
Mechanical properties
Locomotion

Résumé. – Comparaison de la morphologie et des propriétés mécaniques des lépidotriches de gobioides grimpeurs et non grimpeurs d'Hawaii.

De nombreux gobies inféodés aux îles tropicales présentent un cycle de vie amphidrome : les juvéniles viennent de la mer et migrent en eau douce vers les zones en amont. Cependant, dans beaucoup d'îles, les cascades représentent un obstacle à l'atteinte des zones amont. Chez certaines espèces, les juvéniles escaladent ces cascades grâce à une ventouse née de la fusion des nageoires pelviennes. Les adultes de certaines espèces sont aussi capables d'escalader les cascades pour retourner aux habitats en amont après en avoir été délogés par les crues violentes qui peuvent survenir dans les rivières tropicales. Plusieurs éléments structuraux des nageoires ont été examinés afin de déterminer leur rôle dans le succès de l'escalade. Dans cette étude, nous avons comparé la morphologie et la rigidité flexurale des rayons des nageoires caudales et pelviennes, les lépidotriches, pour quatre des cinq espèces natives des rivières hawaiiennes, incluant des espèces grimpeuses et non grimpeuses. Nous avons cherché à évaluer comment la structure et les propriétés mécaniques du squelette des nageoires pouvaient faciliter l'escalade. Les rayons des pelviennes sont plus flexibles que ceux de la nageoire caudale, et ceux des espèces grimpeuses sont plus courts et plus flexibles que ceux des espèces non grimpeuses. Chez les gobies grimpeurs, ces propriétés des rayons pourraient leur permettre de maximiser le différentiel de pression et la force d'adhésion de leur ventouse, sans pour autant avoir une ventouse trop développée qui réduirait l'hydrodynamisme. Ces résultats montrent comment les variations des propriétés mécaniques du squelette peuvent contribuer au développement de capacités fonctionnelles remarquables, comme celle de grimper, et ainsi contribuer à la distribution écologique des poissons dans les cours d'eau insulaires tropicaux.

The streams of tropical Pacific islands present many functional challenges to the fish species that inhabit them. In particular, streams on many of these islands are subject to periodic catastrophic disturbances ranging from flash floods (Fitzsimons and Nishimoto, 1995) to volcanic eruptions (Schoenfuss *et al*., 2004). Many species of gobioid fishes that live in such habitats exhibit a distinctive life history pattern, amphidromy, in which larvae that hatch in freshwater are swept downstream by currents to the ocean, where they develop for a period of several months. After sufficient growth, larvae migrate back to freshwater where they metamorphose and grow to adulthood (McDowall, 2001, 2003, 2004). This life history pattern is thought to provide a population reservoir from which streams can be repopulated after natural disasters (Fitzsimons and Nishimoto, 1995); however, it also subjects island stream gobies to a host of rigorous functional perils (Blob *et al*., 2010; Maie *et al*., 2014; Diamond *et al*., 2016).

Among the perils faced by Pacific island stream gobies is the need, for some species, to climb tall waterfalls that block

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the way to upstream adult habitats (Schoenfuss and Blob, 2003). Such climbing is aided by the presence of an adhesive sucker formed from the fusion of the pelvic fins (Budney and Hall, 2010; Maie *et al*., 2012). However, climbing can be achieved through two distinct mechanisms (Schoenfuss and Blob, 2003). In the Hawaiian Islands, *Awaous stamineus* (Eydoux & Souleyet, 1850) and *Lentipes concolor* (Gill, 1860) both use a mode called "powerburst climbing", in which fish rapidly adduct the pectoral fins and then undulate the body axis side-to-side to propel themselves upwards for a few cycles, until reattaching to the substrate with the pelvic sucker. In contrast, *Sicyopterus stimpsoni* (Gill, 1860) climbs by "inching", alternately detaching and reattaching its pelvic sucker and a second, oral sucker, with little fin or tail motion. Climbing behaviour is a strong source of functional selection in juveniles (Blob *et al*. 2008, 2010; Kawano *et al*., 2013), but is retained by adults of these species, which may use such abilities to re-ascend waterfalls after displacement by elevated pulses of flow (Blob *et al*., 2007; Maie *et al*., 2012).

In addition to differing in climbing mechanisms, gobioid species also differ in their climbing performance. Among the five gobioid species native to the Hawaiian Islands, two do not climb at all. First of these is the piscivorous Hawaiian sleeper, *Eleotris sandwicensis* Vaillant & Sauvage, 1875, in which the pelvic fins remain unfused (Schoenfuss and Blob, 2007). *E. sandwicensis* belongs to a clade that is the outgroup of the remaining native stream fishes, which all belong to the more restricted gobiid clade and exhibit fusion of the pelvic fins into an adhesive disc. The second non-climbing, native Hawaiian stream fish is the goby *Stenogobius hawaiiensis* Watson, 1991, which lives entirely within estuarine downstream reaches below waterfalls (Schoenfuss and Blob, 2007). However, climbing species also differ in their performance. For example, the powerburst climbing goby *Awaous stamineus* shows proficient climbing as a juvenile and reaches upstream distributions above waterfalls (Schoenfuss and Blob, 2003; Blob *et al*., 2006), but tends to lose the ability to climb as it increases to large size (Blob *et al*., 2007). In contrast, the inching goby *Sicyopterus stimpsoni* and the powerburst climbing goby *Lentipes concolor* are both strong climbers as juveniles and maintain this ability into adulthood (Blob *et al*., 2006, 2007). Several traits promote such differences in climbing performance both within and across species, including external body shape, axial muscle fibre proportions, and adhesive capacity (Schoenfuss and Blob, 2003; Blob *et al*., 2006, 2008, 2010; Cediel *et al*., 2008; Schoenfuss *et al*., 2011, 2013; Leonard *et al*., 2012; Maie *et al*., 2012). A variety of components contribute to the performance of the adhesive disc in particular, including its overall size (Maie *et al*., 2007, 2012) and the leverage of the muscles that insert at the base of its fin rays (Maie *et al*., 2013; Schoenfuss *et al*., 2013). However, little is known about the mechanical properties of the lepidotrichia that comprise the fin rays themselves.

Fish have fine control over the position and curvature of individual lepidotrichia via muscles that insert onto the proximal lepidotrichial heads, but do not extend into the main body of the fins themselves (Alben *et al*., 2007). Therefore, performance of a fin is largely determined by the material properties of its lepidotrichia and fin webbing. Given the dynamic movements of the pelvic sucker during attachment and detachment from surfaces in gobies, the performance of this critical structure for climbing could depend considerably on the morphology and flexural stiffness (*i.e.* resistance to bending) of the lepidotrichia that comprise its skeleton (Wicaksono *et al*., 2016). The flexural stiffness of lepidotrichia likely varies not only among fins, but among lepidotrichia within individual fins (Taft, 2011; Chadwell and Ashley-Ross, 2012).

In this study, we compared the morphology and flexural stiffness of the lepidotrichia in the pelvic and caudal fins for four of the five native species of Hawaiian stream fishes: the non-climbing sleeper *E. sandwicensis*, the non-climbing goby *S. hawaiiensis*, the powerburst-climbing goby *A. stamineus*, and the inching goby *S. stimpsoni*. Through these comparisons, we sought to evaluate whether the structure and mechanical properties of lepidotrichia differ between fins that are used in suckers versus those that are not, and whether the structure and mechanical properties of the pelvic and caudal lepidotrichia differ between climbing and non-climbing species. The differences in pelvic function across these taxa provide a basis for predicting differences in the structure and functional performance of their fin rays. With regard to lepidotrichial morphology, we first predict that pelvic lepidotrichia from all gobies will differ in structure from the caudal lepidotrichia, and from the pelvic lepidotrichia of non-climbing sleepers, because of the fusion of pelvic fins into a sucker in gobies. We also predict morphological differences between non-climbing and climbing species. In particular, pelvic lepidotrichia may show the greatest segmentation and branching in climbing species, thereby improving flexibility of the sucker to compress against the substrate during adhesion (Lundberg and Marsh, 1976; Budney and Hall, 2010; Taft and Taft, 2012). Such segmentation and branching might be most pronounced in *S. stimpsoni*, the best climber among our four focus species, though its use of an additional oral sucker might confound this expectation. With regard to flexural stiffness, we predict that lepidotrichia from fins fused into suckers will be more flexible (*i.e.* have lower flexural stiffness) than those from caudal fins; moreover, the suckers of climbing species should have more flexible fin rays than those from non-climbing goby suckers and those from the unfused pelvic fins of eleotrids. Position within the fin may also play an important role. For example, studies of the median fins in bluegill sunfish found that the posterior regions of the fins are more flexible than anterior regions, which has important functional consequences during swimming. Here, we investigate whether the position of lepidotrichia in the fin also matters for the pelvic fins, which are modified for the very different function of suction (Chadwell *et al.*, 2012).

MATERIALS AND METHODS

Fish collection

Specimens from our four focal species (Tab. I) were collected by dipnet on the Big Island of Hawai'i in March 2015 and March 2016. Specimens of *Eleotris sandwicensis* and *Stenogobius hawaiiensis* were collected from Waiakea Pond, and specimens of *Awaous stamineus* and *Sicyopterus stimpsoni* were collected from Hakalau stream. After collection, fish were transported by car, in stream water, to the field station of the Hawai'i Division of Aquatic Resources in Hilo, Hawai'i for use in analyses of swimming performance unrelated to this study. At the completion of swimming performance studies (< 48 h), fish were euthanised in 0.25 g/L neutrally buffered tricaine methanesulfonate (MS-222) solution, photographed, measured, and weighed. Fish were then frozen for at least three days (–20°C) before being shipped overnight, on ice, to lab facilities of the University of Wisconsin-Parkside. Upon arrival, fish specimens were returned to a –20°C freezer until mechanical property tests were conducted.

Morphological specimen preparation and observation

Three individuals from each species were cleared and stained for bone and cartilage according to standard protocols (Song and Parenti, 1995). Digital images of the pelvic and caudal fins were taken from all specimens using a Leica EC3 high-speed digital colour camera and associated acquisition software (Leica LAS EZ software v 3.0) mounted on a stereo microscope (Leica S6 D Greenough). Digital images were acquired from specimens submerged in glycerin. Pelvic and caudal fins were photographed in a neutral resting position, though some were spread slightly to prevent overlap between fin rays. Specimens were illuminated from the top.

Table I. – Summary of whole-body-size measurements for the specimens in the study. Information includes sample size (N), mean, standard deviation (S.D.), minimum (Min.), and maximum (Max.) for body mass and rostrum-to-tail length.

Genus	N	Body mass (g)				Rostrum-to-tail length (cm)			
		Mean	S.D.	Min.	Max.	Mean	S.D.	Min.	Max.
Eleotris	4	25.16	2.05	22.79	27.36	13.50	0.76	12.98	14.60
Stenogobius	4	5.44	1.49	4.40	7.14	9.29	1.02	8.15	10.11
Awaous	4	13.91	5.85	9.02	21.40	10.68	1.36	9.40	12.00
Sicyopterus	3	5.84	1.70	4.63	7.04	8.11	0.44	7.80	8.42

Evaluation of lepidotrichial stiffness

We used three-point bending tests to explore variation in the stiffness (*i.e.* resistance to bending) of a sample of the lepidotrichia from the pelvic and caudal fins of our four focal fish species. Like nearly all biological structures, lepidotrichia are not homogenous. Instead, they are segmented beams that are made up of two hemitrichia joined by collagenous material. Previous studies of a variety of other non-homogenous and/or segmented biological beams suggest that the mechanical properties of such beams closely approximate those of ideal beams (Etnier 2001, 2003). Stiffness of a structure can be quantified through determination of the mechanical property "flexural stiffness," or EI, which is the product of two factors: the elastic modulus (E) of the material and the second moment of area (I) of the item being tested (Vogel, 2003). The elastic modulus (also called "tangent Young's modulus") of a structure reflects the stiffness of its material, whereas the second moment of area of a structure is a geometric measurement of its shape that reflects the distribution of material about its cross-section (*ibid.*). Consideration of both factors is particularly appropriate for evaluations of mechanical properties for non-homogenous structures (*ibid.*). Individual specimens of fish were thawed on the same day that they were tested, and kept in distilled water at room temperature between trials. We extracted lepidotrichia from four individual *A. stamineus*, *E. sandwicensis* and *S. hawaiiensis*, and three individual *S. stimpsoni* (Tab. I). These sample sizes, although from a limited number of individuals, are comparable to those used to characterize bone mechanical properties in many comparative studies (*e.g.* Currey, 1979; Blob and LaBarbera, 2001; Horton and Summers, 2009).

Given the small size of our specimens and their nearly round cross-sections, we did not attempt to obtain exact measurements of cross-sectional geometry to calculate I values for our determinations of flexural stiffness. Instead, we measured the diameters of our lepidotrichia specimens and calculated I under the assumption that the fin rays were solid cylinders. Based on previous analyses, we know that lepidotrichia are more like hollow cylindrical tubes than solid cylinders (Geerlink and Videler, 1987; Alben *et al.*, 2007; Taft and Taft, 2012). However, I is virtually the same for a solid cylinder and a hollow tube in which the ratio of the radius of the outer circle (bone) to the radius of the inner cylinder (collagen) is 1:1. Based on previous studies of fin ray morphology (Budney and Hall, 2010; Taft, 2011), we felt that the assumption that the ratio of the outer and inner radii is close to 1:1 was reasonable. Therefore, the assumption that lepidotrichia are solid cylinders would not substantially misestimate the stiffness of these beams.

We tested all of the segmented pelvic lepidotrichia from each individual, but not the anterior pelvic spine

Table II. – Summary of measurements of the lepidotrichia for the specimens in the study. Information includes sample size (N), mean, standard deviation (S.D.), minimum (Min.), and maximum (Max.) for lepidotrichium length and diameter, and for flexural stiffness, mean, upper and lower bound of the 95% confidence interval of the mean (95% Conf. Int.), minimum (Min.), and maximum (Max.).

Genus	Fin	N	Ray length (mm)				Ray diameter (mm)				Flexural stiffness (N × mm^2)				
			Mean	S.D.	Min.	Max.	Mean	S.D.	Min.	Max.	Mean	95% Conf. Int.		Min.	Max.
Eleotris	Pelvic	21	14.12	3.67	6.80	20.68	0.27	0.07	0.10	0.37	0.009	0.007	0.011	0.0035	0.0338
	Caudal	10	22.96	2.08	20.61	27.69	0.38	0.05	0.27	0.44	0.025	0.019	0.034	0.0149	0.0529
Stenogobius	Pelvic	15	11.13	3.72	4.32	16.43	0.14	0.05	0.05	0.26	0.012	0.008	0.016	0.0030	0.0444
	Caudal	11	19.75	4.00	13.63	24.26	0.17	0.07	0.07	0.27	0.018	0.012	0.028	0.0087	0.0556
Awaous	Pelvic	20	10.69	2.20	6.67	14.47	0.20	0.06	0.11	0.37	0.006	0.003	0.012	0.0002	0.3096
	Caudal	12	20.04	3.00	14.14	23.89	0.38	0.11	0.25	0.62	0.011	0.007	0.016	0.0000	0.0300
Sicyopterus	Pelvic	10	5.50	0.90	4.30	6.81	0.17	0.02	0.14	0.21	0.004	0.002	0.007	0.0011	0.0151
	Caudal	8	14.55	2.34	10.17	16.89	0.20	0.07	0.12	0.31	0.008	0.006	0.010	0.0046	0.0117

(Tab. II). To provide a comparison to the potentially specialized fin rays of the pelvic sucker, we also sampled three caudal lepidotrichia from each individual: one dorsal, one middle and one ventral (Tab. II). To access specimens for testing, lepidotrichia were dissected from the caudal fins and the left pelvic fin unless the left fin was damaged. Lepidotrichia were prepared from one fin at a time immediately prior to testing and were left as intact as possible, with skin and collagenous attachments undisturbed.

A modified three-point bending test (Horton and Summers, 2009) was used to generate stress-strain curves for individual lepidotrichia. Tests were performed with an Instron 5942 MicroTester with a 5N load cell. Rays were placed in a custom-made fixture with an adjustable span length. Before each trial, the span width between the two supporting load points was set to 20% of the total length of each individual fin ray. Individual lepidotrichia were placed across these supports. Lepidotrichia were oriented with the hemitrichia on top of one another. The slight natural curvature of the fin rays ensured that each specimen and location was tested in the same anatomical orientation, with the slightly concave surface of each ray directed towards the central load point. Each lepidotrichium was point loaded in the exact calculated middle (50% of the distance from the proximal head of the lepidotrichia) of each ray. For all rays tested, the middle of the fin ray was included in the segmented region of the ray, and in most cases this location was distal to at least the first branching point. All lepidotrichia were loaded at a rate of 4 mm/min. Each lepidotrichium was loaded only once to minimize the risk for microfracture or breakage, unless they rolled during testing (which happened rarely). Rays were loaded until they reached a force plateau. The total displacement varied among rays, but total deformation was always less than 10% of the total length of the ray. Displacing the lepidotrichia less than 10% of their total length minimizes deformation due to shear (Blob and LaBarbera, 2001). Breakage during testing was extremely rare, but any lepidotrichia that showed any evidence of cracks or breakage were excluded from the analysis.

To evaluate and compare the stiffness of fin ray specimens, we used the “automatic modulus” calculation available in Bluehill 3 software (Illinois Tool Works Inc., Norwood, MA) to calculate the steepest part of the slope of the stress/strain curve resulting from the deformation of the lepidotrichia that are point-loaded in the middle of their span. The true Young’s modulus of elasticity would be the slope of the initial portion of the stress/strain curve. We measured the steepest part of the slope, or a tangent Young’s modulus (*i.e.* elastic modulus, or *E*), which is a measure of the maximum stiffness of the material (Vogel, 2003). The automatic modulus algorithm we used searches the data between the first data point that is greater or equal to 2% of the maximum load value. Then, it performs a zero-slope yield calculation to determine whether there is a yield point in the data. Next, the algorithm uses the first data point as the start value, and the yield point or maximum load as the end value, whichever happens first. The data on the strain axis between the start and end values are divided into 6 equal regions with 0% overlap. The program then applies a least-squares fit algorithm to all points in each of the six regions to determine their slopes. Next, it determines the pair of consecutive regions that have the highest slope sum. Finally, it determines which of these two regions has the highest slope, and assigns the modulus to that region.

Statistical analyses

We used a mixed linear model approach to evaluate several hypotheses. The random effects in all models were individual and fin nested within individual to account for variation in fin size or shape among individuals. We included three covariates in our models: mass (g) of each individual, the position of the lepidotrichia in the fin (anterior to posterior in the pelvic fin, dorsal to ventral in the caudal fin) and position in the fin squared. This third covariate allowed the middle of the fins to be different than the edges, instead

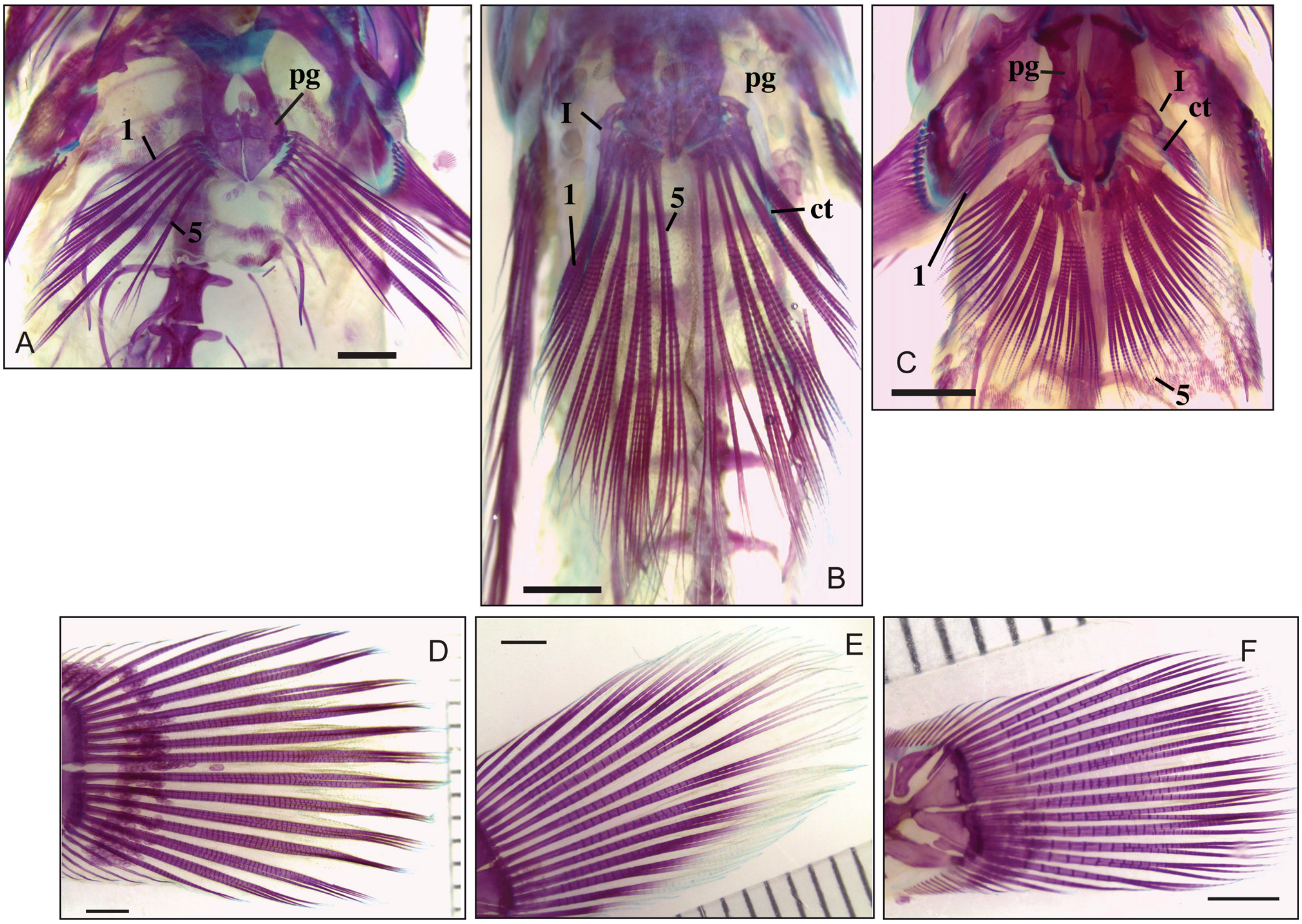

Figure 1. – Ventral view of the skeletal anatomy of the pelvic fins and girdles (**A-C**) and lateral view of caudal fins (**D-F**) in cleared and stained specimens of *Eleotris sandwicensis* (A, D), *Stenogobius hawaiiensis* (B, E), and *Sicyopterus stimpsoni* (C, F). For specimens A-C, the rostral end of the specimen is at the top, caudal toward the bottom. For specimens D-F, the dorsal part of the tail is toward the top of the photo, the ventral portion of the fin is toward the bottom. Abbreviations are as follows: I = spine, rays 1 (most anterior) and 5 (most posterior) are labeled, pg = pelvic girdle, ct = cartilaginous tip of anterior spine. Scale bar in all photos is 2 mm.

of varying in just the dorsal to ventral direction. We were interested in the following potential fixed effects: climbing (versus non-climbing), fin (pelvic versus caudal), genus (*Eleotris, Stenogobius, Awaous, Sicyopterus*), and the two interactions: fin × climbing or fin × genus. We also included interactions between any one covariate and any of the fixed effects (*e.g.* genus × position, fin × mass or genus × fin × position).

We used a model-testing procedure to choose which combination of effects best-explained the observed data, comparing both the Corrected Akaike's and Bayes' Information Criterion among all models, and choosing the model with the lowest AIC and BIC scores. The models with similar AIC values mostly added Mass as a covariate. Inspection of the coefficients of the models that included Mass found that Mass itself had a very small effect, and that the coefficients of the other terms hardly changed at all. This suggested that Mass was not a valuable addition to the model, a suggestion that was borne out by the large penalty to BIC found among these models. The one model with a similar BIC value to the selected model was a simpler model that excluded the effect of position within pelvic fin, but also had a substantially worse AICc. The models ranged from a random-effect-only model to one that included all four fixed effects. After designating one of the models as the best among our options, we used a bootstrap function to calculate confidence intervals for that model's fixed effects. There were 496 possible models. The remaining model with the lowest AICc was considered to be the model that best explains our observed variation in the flexural stiffness of fin rays from Hawaiian gobioid fishes (Aho *et al.*, 2014) (Tabs III, IV).

Table III. – Relative support for mixed linear models of the flexural stiffness of caudal and pelvic lepidotrichia of fish from the genera *Eleotris* (N = 4), *Stenogobius* (N = 4), *Awaous* (N = 4), and *Sicyopterus* (N = 3). The data set included 107 rays in total. Model measures include log-Likelihood (log L), degrees of freedom (df), Akaike (AIC) and Bayes' (BIC) Information Criteria, corrected AIC (AICc), and difference in AICc from the best model (dAICc). The model with the best (smallest) AICc is in bold. The depicted models all have AICc greater than the null model, as well as the greatest likelihood for their degrees of freedom are shown. Random effects in the models all included Individual and Fin nested within Individual.

Model	log L	df	AIC	BIC	AICc	dAICc
Null	–53.1	4	114.3	124.9	114.6	26.1
Fin	–45.8	5	101.7	115.1	102.3	13.8
Fin + Climbs	–40.6	6	93.3	109.3	94.1	5.6
Mass + Fin + Climbs	–40.4	7	94.7	113.4	95.9	7.3
Fin + Climbs + Fin:Position	**–35.5**	**8**	**87.0**	**108.4**	**88.5**	**0.0**
Mass + Fin + Climbs + Fin:Position	–35.3	9	88.6	112.7	90.5	2.0
Fin + Genus + Fin:Position	–34.3	10	88.6	115.3	90.9	2.4
Mass + Fin + Genus + Fin:Position	–33.8	11	89.7	119.1	92.5	4.0
Fin + Genus + Fin:Position + Fin:Position^2	–33.4	12	90.8	122.9	94.1	5.6
Mass + Fin + Genus + Fin:Position + Fin:Position^2	–33.0	13	91.9	126.7	95.9	7.3
Mass + Genus + Fin:Genus + Fin:Position	–32.5	14	93.0	130.5	97.6	9.1
Fin + Fin:Genus + Fin:Position + Fin:Position^2	–32.0	15	94.1	134.2	99.3	10.8
Mass + Fin + Fin:Genus + Fin:Position + Fin:Position^2	–31.6	16	95.1	137.9	101.1	12.6
Mass + Fin + Genus + Fin:Genus:Position	–33.0	17	100.0	145.4	106.8	18.3
Fin + Genus + Fin:Position + Fin:Genus:Position^2	–31.8	18	99.6	147.7	107.4	18.8
Mass + Fin + Genus + Fin:Position + Fin:Genus:Position^2	–31.4	19	100.8	151.6	109.5	21.0
Mass + Fin + Fin:Genus + Fin:Genus:Position	–31.8	20	103.5	157.0	113.3	24.8
Fin + Fin:Genus + Fin:Position + Fin:Genus:Position^2	–30.2	21	102.4	158.6	113.3	24.8

Table IV. – Effect estimates for the model with the lowest AICc in the study (see Tab. III). Two different degrees of freedom were used to compute p-values from the t-statistics: 2, which corresponds to using the smallest number of individuals per genus as the sample size, and 14, which corresponds to using the number of individuals in the sample as the sample size. Standard deviations of random effects: Individual (N = 15): 0.0997, Fin (N = 30): 0.0000, Residual (N = 107): 0.3254.

Effect	Estimate	Std. error	*t*	p (2 d.f.)	p (14 d.f.)
Intercept	–2.01112	0.07140	–28.17	0.0006	< 0.0001
Pelvic	–0.30009	0.06521	–4.602	0.0221	0.0002
Non-Climber	0.31468	0.08179	3.848	0.0307	0.0009
Caudal:Position	0.00329	0.10716	0.031	0.4890	0.4879
Pelvic:Position	0.20330	0.06200	3.279	0.0409	0.0027

RESULTS

Lepidotrichial morphology

All of the individual lepidotrichia that support the pelvic fins in the Hawaiian stream fish that we tested are unsegmented and unbranched proximally, but become segmented and branched distally (Fig. 1). One common exception to this pattern is that the lepidotrichia at the edges of the fins can remain unbranched. These conditions are common among teleosts, although in some species the fin rays remain unbranched for their entire length, particularly those that are used to directly contact the substrate (Taft, 2011). The lepidotrichia articulate proximally with a cartilaginous pad that is contiguous and distal to the bony pelvic girdle. Muscles that control the relative position and curvature of the lepidotrichia originate on the pelvic girdle and insert onto the proximal heads of each hemitrichia, one dorsal, one ventral. Lepidotrichia are controlled by muscles that originate on the pelvic girdle and insert directly onto the proximal surface of the hemitrichia. Each lepidotrichium therefore has separate sets of muscles, dorsal and ventral, that control the relative position and curvature of the individual rays (Winterbottom, 1974).

In *E. sandwicensis*, the pelvic fins are separate and not modified for suction. Each pelvic fin is supported by six-segmented lepidotrichia, with all but the most anterior of these rays showing distal branching (Fig. 1A). The individual segments of each lepidotrichium are quite short, shorter than the rays are wide, which is similar to the condition found in the gobiid taxa we studied (Fig. 1B, C). There are fewer branches distally in *E. sandwicensis* compared with both *S. hawaiiensis* and *S. stimpsoni*, giving the rays a thinner, more distally streamlined appearance than those of the gobies (Fig. 1A-C).

In the remaining three goby species we examined, the pelvic fin rays are joined together anteriorly and distally by a membrane that encloses the cavity used for suction, the frenum (Budney and Hall, 2010). This membrane is not clearly visible in photographs of cleared and stained specimens. The anterior portion of this membrane is supported by an anterior pelvic spine that is not present in *E. sandwicensis*. This spine has two components, a proximal unsegmented region and a cartilaginous distal tip that is similar to that described previously for other species of gobies (*ibid.*). In *S. hawaiiensis*, the anterior spine is relatively longer and straighter than in *S. stimpsoni* (Fig. 1B, C); however, in both species, the relative length of the cartilaginous tip is slightly shorter than that of the bony proximal region.

The pelvic fin rays of the non-climbing *S. hawaiiensis* are longer and have fewer distal branching points than those of the climbers, *A. stamineus* and *S. stimpsoni*. Lepidotrichia of *S. hawaiiensis* most closely resemble previous descriptions of *Awaous tajasica* (Lichtenstein, 1822) (Budney and Hall, 2010). The individual lepidotrichia of *S. hawaiiensis* are longer than those of *S. stimpsoni* and *A. stamineus* (not shown, but similar to *S. stimpsoni*), and the pelvic sucker therefore has a more elongate, rounded triangular shape (see also Maie *et al.*, 2012, 2013). In contrast, the pelvic discs of *S. stimpsoni* and also *A. stamineus* (not shown) more closely resemble those of previous descriptions of *Tukugobius phillipinus* (Herre, 1927) (Budney and Hall, 2010). The individual rays are relatively shorter in *S. stimpsoni* than in *S. hawaiiensis*, resulting in a more rounded suction disc; however, the unsegmented proximal region of each pelvic fin ray in *S. stimpsoni* is relatively wider and more robust than in *S. hawaiiensis*. The dorsal hemitrichia of *S. stimpsoni* have dorsally projecting flanges that provide a robust site for attachment of the dorsal muscles. The ventral hemitrichia of *S. stimpsoni* also have a much more robust attachment site for the ventral muscles, although they lack the more extreme flanges. Another difference between the lepidotrichia of *S. stimpsoni* and *S. hawaiiensis* is that those of *S. stimpsoni* are more branched distally, which may enhance support for the edges of the pelvic sucker.

The caudal fins of all species examined are much less variable than the pelvic fins. This is not surprising given the varying degrees of pelvic fin specialization for suction in gobies. All four of the Hawaiian stream species examined have bluntly rounded tails. The principal caudal lepidotrichia are defined as the segmented, branched lepidotrichia, as well as one unbranched lepidotrichium immediately dorsal and ventral to the branched rays (Schultze and Arratia, 1989). All of the principal caudal lepidotrichia articulate proximally with the hypural bones. The principal lepidotrichia in all species become branched at about halfway to two-thirds of the distance from the proximal head of the ray. Interestingly, in *S. hawaiiensis* the distal tips of the caudal lepidotrichia are not as well ossified as in *E. sandwicensis* or *S. stimpsoni*, a pattern observed in the pelvic lepidotrichia as well.

Flexural stiffness

The model that best explains the variation in flexural stiffness included the fixed effects of climbing ability, fin type, and the position of the lepidotrichia within the fin (Tab. III). This means that the lepidotrichia of the climbing species have significantly lower flexural stiffness than species that do not climb (Fig. 2). However, among all species, the caudal lepidotrichia have a higher flexural stiffness than the pelvic lepidotrichia (Fig. 2). When we compare the pelvic and caudal fins, position of the lepidotrichia does affect the stiffness in pelvic fins, but not in caudal fins. In pelvic fins, the more posterior lepidotrichia (in the middle of the disc) are stiffer than more anterior lepidotrichia (on the outer edges of the disc).

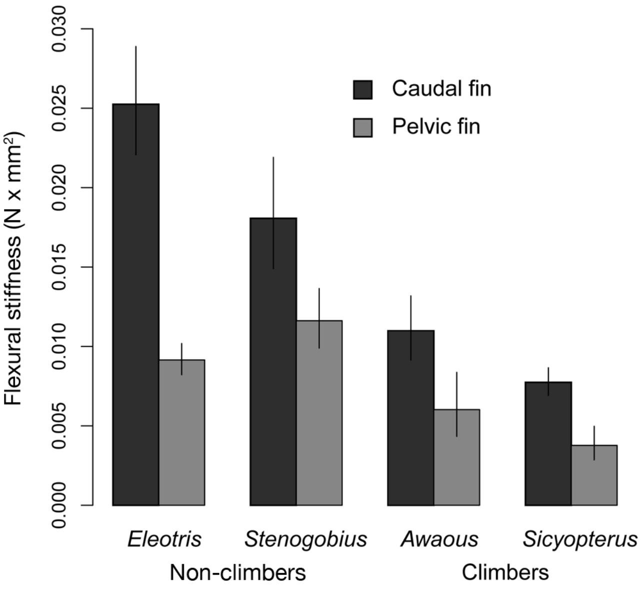

Figure 2. – Mean flexural stiffness (N × mm^2) of the fin rays of the caudal fins (dark gray) and pelvic fins (light gray) of non-climbing and climbing gobioid species. Standard errors are indicated by the vertical lines. See Table II for sample sizes.

DISCUSSION

Our results show that specializations of the pelvic fins of climbing gobies include not only features of shape, size, and muscular arrangements (Maie *et al.*, 2007, 2012, 2013; Schoenfuss *et al.*, 2013), but also extend to the mechanical properties of the fin ray skeleton. The distinctiveness of the mechanical properties of climbing goby lepidotrichia is evident through comparisons of stiffness among fins used primarily for climbing (pelvic fins) and those used primarily for swimming (caudal fin). Pelvic lepidotrichia tend to be more flexible than caudal lepidotrichia (Fig. 2), despite the broad similarity in construction of both types of elements. These patterns are congruent with our previous findings that the suckers also show patterns of allometric growth that are distinct from those of the caudal fin (Maie *et al.*, 2007). It is possible that higher stiffness of the caudal lepidotrichia may provide advantages for the role of the tail in swimming propulsion, helping the tail to be sufficiently stiff to transfer momentum to the surrounding water with minimal dissipation.

However, given the conservative structure of the tail across gobioid taxa (Fig. 1 D-F), it is likely that the primary novelty in lepidotrichial properties is present in the rays of the pelvic fins. This is particularly suggested because the shortest, most flexible lepidotrichia are found in the pelvic suckers of climbing gobies (Fig. 2). In these species, short fin rays would reduce drag on the sucker (Blob *et al.*, 2008, 2010), but fin ray flexibility would convey the ability to be pressed against a surface, and subsequently be maximally retracted by muscles to produce the greatest possible volume to be enclosed within the fin (Maie *et al.,* 2012). This retraction would decrease the pressure within this space to maximize its differential compared to the surrounding ambient environment, thereby elevating adhesive strength (*ibid.*). Thus, the combination of short, but flexible, lepidotrichia in goby pelvic suckers circumvents a functional tradeoff for large sucker size between increasing advantageous adhesive strength but also increasing undesirable hydrodynamic drag that could dislodge climbing fish. Instead, short, flexible fin rays facilitate the maximization of adhesive strength despite small sucker size. Also, as suggested for tree-climbing species of mudskipper (Wicaksono *et al.,* 2016) flexible fin rays may be better in moulding to the shape of an uneven substrate, thereby helping to maintain a tighter adhesive seal.

Identification of functionally related specialization in the mechanical properties of gobioid lepidotrichia provides further evidence that variation in skeletal mechanical properties can contribute to the adaptations of organisms to distinctive environments and functional demands (Currey, 1979; Blob and Snelgrove, 2006; Blob *et al.*, 2014; Kawano *et al.*, 2016). Given the dramatic ecological demands faced by many species of Pacific island stream gobies, it is, perhaps, not surprising that their functional adaptations should pervade numerous body systems. With such extensive possibilities for examination, comparative studies of functional performance in Pacific stream fishes hold tremendous promise for future studies that will improve understanding of species distribution and evolution in these distinctive island ecosystems.

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